The Mechanism Underlying T Cell Help for Induction of an Antigen-Specific In Vivo Humoral Immune Response to Intact *Streptococcus pneumoniae* Is Dependent on the Type of Antigen[1]

Zheng-Qi Wu,* Yi Shen,* Abdul Q. Khan,* Ching-Liang Chu,† Richard Riese,‡ Harold A. Chapman,† Osami Kanagawa,§ and Clifford M. Snapper[2]*

Little is known concerning the role of T cells in regulating an anti-polysaccharide Ig response to an intact pathogen. We previously reported that the in vivo Ig responses to *Streptococcus pneumoniae* (strain R36A), specific for pneumococcal surface protein A (PspA) and for the phosphorylcholine (PC) determinant of C-polysaccharide, were both dependent on TCR-$\alpha\beta^+$ T cells and B7-dependent costimulation, although only PspA-specific memory was generated. In this report, we show that the T cell help underlying these two Ag-specific Ig responses is distinct. Using H-Y-specific T cell transgenic mice made "nonleaky" by crossing with mice genetically deficient for TCR-α, we demonstrate that the T cell help for the anti-PC, in contrast to the anti-PspA, response is TCR-nonspecific and occurs normally in the absence of germinal center formation, although it is still dependent on B7-dependent costimulation. Consistent with these data, we demonstrate, using cathepsin $S^{-/-}$ mice, that although the anti-PC response is largely dependent on $CD4^+$ T cells, there is a reduced (or lack of) dependence, relative to the anti-PspA response, on the generation of new peptide-MHC class II complexes. In this regard, the T cell help for an optimal anti-PC response is delivered more rapidly than that required for an optimal anti-PspA response. Collectively, these data demonstrate a novel accelerated TCR-nonspecific B7-dependent form of T cell help for augmenting a polysaccharide-specific Ig response to an intact bacterium without the generation of memory. ***The Journal of Immunology*, 2002, 168: 5551–5557.**

Immunoglobulin isotype responses specific for protein and polysaccharide Ags are induced through distinct mechanisms (1, 2). This is due, in part, to the ability of proteins, but not polysaccharides, to recruit cognate $CD4^+$ T cell help through TCR recognition of peptide-MHC class II complexes on the surface of APCs (3). In contrast to proteins, polysaccharides express regularly spaced repeating epitopes that induce multivalent membrane Ig (mIg)[3] cross-linking on the B cell surface. This mediates potent mIg-dependent B cell signaling (4) that, by itself, induces only B cell proliferation, but in concert with cytokines and/or polyclonal microbial activators, can costimulate Ig secretion and Ig class switching (5, 6). Although Ig responses to polysaccharides can occur in a T cell-independent manner, these responses may be regulated by T cells, as well as non-T cells (1, 2).

In vitro studies suggest a potential role for non-T cells, such as dendritic cells (DC), macrophages, and/or NK cells, in providing B cell help for an anti-polysaccharide response through their release of cytokines and/or their membrane expression of B cell activating ligands (6–11). Some in vivo studies demonstrate that NK cells can regulate Ig isotype selection (12, 13), and that APCs, through their release of B lymphocyte stimulator, may stimulate B cell maturation in response to T cell-independent Ags (14, 15). Although polysaccharides fail to associate with MHC molecules (3) and hence cannot directly recruit cognate T cell help, their coexpression with proteins by an intact pathogen could in theory mediate cognate $CD4^+$ T cell help for polysaccharide-specific B cells, similar to the mechanism underlying the effectiveness of soluble protein-polysaccharide conjugate vaccines (16, 17). In this regard, it was recently demonstrated that blocking CD40-CD40-ligand interactions during an in vivo response to intact *Streptococcus pneumoniae*, using an anti-CD40-ligand mAb, inhibited induction of Ig specific for capsular, but not cell wall, polysaccharide. This suggested a possible role for cognate T cell help for the anti-capsular polysaccharide response (18). In contrast, anti-CD40-ligand mAb has no effect on the in vivo trinitrophenyl (TNP)-specific Ig response to the soluble T cell-independent Ag, trinitrophenyl-Ficoll (19). A number of additional mechanisms have been proposed whereby T cells could potentially regulate anti-polysaccharide Ig responses, although there is no published data that these are operative during an in vivo response to an intact pathogen (1).

Early in vivo studies demonstrated that T cells could regulate anti-polysaccharide responses in either a positive or negative manner, or influence B cell repertoire or Ig isotype selection (1, 20–25). In addition, it has been shown that an in vivo anti-polysaccharide response to an intact bacteria is diminished in

*Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814; †Pulmonary and Critical Care Division, Department of Medicine, University of California, San Francisco, CA 94143; ‡Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; and §Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

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[2] Address correspondence and reprint requests to Dr. Clifford M. Snapper, Department of Pathology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814. E-mail address: csnapper@usuhs.mil

[3] Abbreviations used in this paper: mIg, membrane Ig; PspA, pneumococcal surface protein A; PC, phosphorylcholine; KLH, keyhole limpet hemocyanin; DC, dendritic cell.

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athymic nude mice, which have a congenital deficiency in T cells (26, 27). Although collectively these studies demonstrated diverse roles for T cells in regulating anti-polysaccharide responses, the majority of these used purified soluble polysaccharides, with or without haptenation, that may not reflect physiologic polysaccharide-specific Ig responses to a biochemically diverse intact pathogen. Further, these studies did not elucidate the underlying mechanism(s) for these T cell effects. In particular, the central question of whether T cells regulate in vivo anti-polysaccharide Ig responses to intact pathogens in a cognate or noncognate manner has not yet been answered.

In a previous study of the in vivo humoral response to intact *S. pneumoniae* (strain R36A), we demonstrated a requirement for TCR-$\alpha\beta^+$ T cells and B7-dependent costimulation for stimulating IgG responses specific for both the cell wall protein, pneumococcal surface protein A (PspA), and the phosphorylcholine (PC) determinant of the cell wall C-polysaccharide (teichoic acid) (28, 29). Of interest, primary immunization with R36A generated little, if any, PC-specific IgG memory, whereas the anti-PspA response in R36A-primed mice was boosted 10- to 20-fold in response to secondary immunization. In addition, the IgG anti-PC response occurred with significantly more rapid kinetics than that observed for induction of PspA-specific IgG. Collectively, these data suggested the possibility that T cells might play distinct roles in these two responses. In the current study, we confirm this hypothesis and provide strong evidence that unlike the T cell help for the anti-PspA response which is cognate, the T cell help for the anti-PC response is TCR-nonspecific.

Materials and Methods

Mice

H-Y $\alpha^{-/-}$ mice were created through introduction of the H-Y (male Ag) TCR transgene into TCR-$\alpha^{-/-}$ mice as previously described (30). Cathepsin S$^{-/-}$ mice were generated as previously described through deletion of the cathepsin S gene exon 5 which contains the active site cysteine of murine cathepsin S and were bred greater than nine generations into the C57BL/6 background (31). B6,129 F_2 mice (The Jackson Laboratory, Bar Harbor, ME) were used as controls for H-Y $\alpha^{-/-}$ mice, and C57BL/6 mice (The Jackson Laboratory) were used as controls for cathepsin S$^{-/-}$ mice. Athymic nude and control BALB/c mice were purchased from NCI (Frederick, MD). All mice were used between 6 and 10 wk of age.

Reagents

PC-BSA and PC-keyhole limpet hemocyanin (KLH), kind gifts of Dr. A. Lees (Biosynexus, Rockville, MD), were synthesized as described previously (28). rPspA, a kind gift of Dr. L. Grinberg (Biosynexus) was expressed in *Saccharomyces cerevisiae* BJ3505 as a His$_6$-tagged fusion protein, and purified by Ni-NTA affinity chromatography (28). The expressed protein includes aa 4–299 of the mature protein. Murine CTLA4Ig and a control fusion protein, L6 was prepared as previously described (32). Rat IgG2b anti-mouse CD4 mAb (GK1.5) and rat IgG2b anti-mouse CD8 mAb (2.43) were purified from ascites by ammonium sulfate precipitation and passage over a protein G column. Purified rat IgG was purchased from Accurate Chemical and Scientific (Westbury, NY). FITC-hamster IgG1κ anti-murine CD3ϵ mAb (clone 145-2C11), PE-rat IgG2bκ anti-murine CD4 mAb (clone GK1.5), and PE-rat IgG2aκ anti-murine CD8 mAb (clone 5.3-6.7) (BD PharMingen, San Diego, CA) were used for flow cytometry.

Preparation of, and immunization with, R36A

A nonencapsulated variant (strain R36A) of virulent *S. pneumoniae* capsular type 2 (strain D39) (33) was grown in Todd-Hewitt broth to mid-log phase and stored at −70°C. For immunization, frozen bacteria were thawed and subcultured on blood agar plates. One to two characteristic colonies were selected and suspended in 200 ml of Todd-Hewitt broth, placed in a shaker water bath at 37°C for 4–6 h until an OD (absorbance at 650 nm) of 0.6 was achieved as measured by a spectrophotometer (Spectronic 100; Bausch & Lomb, Rochester, NY). The 200-ml prep of R36A was then heat-killed by incubation in a 60°C water bath for 10 h (1 h/20 ml). Sterility was confirmed by culture. This bacterial stock, containing 1×10^9 CFU/ml, was aliquoted and frozen at −70°C until used for immunization. Mice were immunized i.p. with 2×10^8 CFU R36A in 250 μl of PBS. Serum samples for measurement of anti-PC and anti-PspA Ab titers were prepared from blood obtained through the tail vein.

Measurement of serum Ag-specific Ig isotype titers

Immulon 2 plates were coated with PC-KLH or PC-BSA (5 μg/ml) and Immulon 4 plates were coated with PspA (5 μg/ml) in 1× PBS for 1 h at 37°C or overnight at 4°C. Plates were then blocked with blocking buffer (1× PBS + 0.5% BSA) at 37°C for 30 min or 4°C overnight. Three-fold dilutions of serum samples in blocking buffer were then added starting at a 1/50 serum dilution. After a 1 h incubation at 37°C, plates were washed thee times with PBT (1× PBS + 0.1% Tween 20). Alkaline phosphatase-conjugated polyclonal goat anti-mouse IgM, IgG3, IgG1, IgG2b, and IgG2a Abs (200 ng/ml final concentration in blocking buffer) were then added and plates were incubated for 37°C for 1 h. Plates were washed five times with PBT. Substrate (4-methylumbelliferyl phosphate) was then added (50 μg/ml, 50 μl/well) and fluorescence was read on MicroFLUOR ELISA reader (Dynatech Laboratories, Chantilly, VA).

Magnetic cell sorting

Magnetic cell sorting was performed according to manufacturer's instructions (Miltenyi Biotec, Auburn, CA). Briefly, spleens were removed aseptically and spleen cells were suspended in cold HBSS + 3% FCS (BioWhittaker, Walkersville, MD). RBCs were lysed with ACK lysing buffer (BioWhittaker). Cells were then suspended for 20 min in sorting buffer (Dulbecco's PBS + 0.5% BSA + 2 mM EDTA) at 1×10^8 cells/ml in the presence of 100 μl/ml of either mouse CD8a (Ly-2) or mouse CD4 (L3T4) MicroBeads (Miltenyi Biotec). Cells were then washed in 20 volumes of sorting buffer to remove unattached MicroBeads and resuspended into sorting buffer (1×10^9 cells/3 ml). Cells were then loaded onto an LS MACS separation column (Miltenyi Biotec) on a MidiMACS magnetic separator. Cells retained in the column were washed, the column was removed from the separator, and then flushed with sorting buffer to recover retained cells. Cell purities were determined by flow cytometry and typically demonstrated purities of 95–99% for both CD4$^+$ and CD8$^+$ T cells.

Adoptive transfer

Two or 6×10^6 BALB/c-derived CD4$^+$ or CD8$^+$ T cells obtained by magnetic cell sorting were suspended in 0.4 ml PBS in the presence of 1 mg of anti-CD8 or anti-CD4 mAb, respectively, and injected i.v. into athymic nude mice. Mice were then challenged 16 h later with R36A. Serum was obtained on day 7 for anti-PC titers and on day 14 for anti-PspA titers.

Quantitation of germinal centers by immunohistochemistry

The staining procedure for detection of germinal centers has been described previously. Briefly, spleens were removed from unimmunized mice and from mice 14 days after i.p. immunization with R36A. Sections (8 μm) were cut, fixed in acetone for 10 min, and stored at −70°C. Germinal center B cells were stained with 15 μg/ml of FITC-peanut agglutinin (ICN Pharmaceuticals, Costa Mesa, CA) for 1 h at room temperature. Slides were washed with PBS, allowed to dry, then covered with Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL) for analysis by fluorescence microscopy using a Zeiss Axiophot fluorescence microscope (Oberkochen, Germany).

Statistics

Data is expressed as the arithmetic mean of Ig titers of individual serum samples ± SEM. Differences between treatment groups were considered significant at $p < 0.05$ using the Student *t* test.

Results

The T cell help required for an optimal IgG anti-PC, relative to IgG anti-PspA, response is delivered more rapidly

We previously demonstrated that the IgG anti-PspA and IgG anti-PC response to R36A was dependent upon TCR-$\alpha\beta^+$ T cells and B7-dependent costimulation (28). A subsequent study indicated that a shorter period of costimulation was required for an optimal anti-PC, relative to an optimal primary anti-PspA response (29). In this regard, we wished to determine whether this was associated with a requirement for a shorter period of T cell help to mediate the anti-PC response. To accomplish this, we acutely depleted T cells on different days (−1, 0, 1, 3, 5, and 7) after R36A immunization

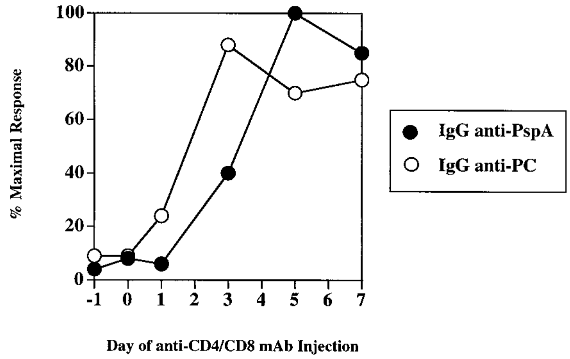

FIGURE 1. The T cell help required for an optimal IgG anti-PC response, relative to IgG anti-PspA, is delivered more rapidly. Different groups of C57BL/6 mice (five mice per group) were injected i.v. with 1 mg each of anti-CD4 and anti-CD8 mAbs at various times relative to i.p. immunization with R36A. Sera were obtained on days 7 (anti-PC) and 14 (anti-PspA) for determination of Ag-specific IgG titers by ELISA. Data are expressed as a percentage of maximal response (i.e., mice receiving 2 mg of control rat IgG followed 1 day later by immunization with R36A). One of two representative experiments.

using a combination of anti-CD4 + anti-CD8 mAbs and measured serum titers of IgG anti-PC and IgG anti-PspA on days 7 and 14, respectively, which are the times at which optimal Ig titers are observed (28, 29) (Fig. 1). A similar dose of total rat IgG was injected on day −1 as a control. Injection of T cell-depleting mAbs 1 day after R36A immunization resulted in a significantly higher IgG anti-PC response to R36A relative to that obtained when mAbs were injected on days −1 or 0, whereas in distinct contrast, there was still a complete inhibition in induction of PspA-specific IgG titers. Injection of mAbs 3 days after R36A immunization had no significant effect on the IgG anti-PC response but was still associated with a significant, though partial, inhibition of the IgG anti-PspA response. Injection of mAb on day 5 had no effect on either response. These data indicate that a shorter period of T cell help is sufficient for mediating an optimal IgG anti-PC vs anti-PspA response and is consistent with our previous observation of a comparably reduced time requirement for B7-dependent costimulation (29).

CD4$^+$ T cells are more efficient than CD8$^+$ T cells for stimulating an anti-PC response to R36A

In a previous study we observed that injection of anti-CD4 mAb alone was sufficient for complete inhibition of the IgG anti-PspA response to R36A (28). In contrast, maximal inhibition of the IgG anti-PC response required injection of both anti-CD4 + anti-CD8 mAbs. Although in vitro studies have demonstrated that CD8$^+$ T cells can stimulate Ig induction by B cells, they were less effective than CD4$^+$ T cells, perhaps in part due to lower CD40 ligand expression (34). To better define the relative contributions of CD4$^+$ and CD8$^+$ T cells for mediating help for the IgG anti-PC response to R36A, we purified splenic CD4$^+$ and CD8$^+$ T cells from naive mice and adoptively transferred them (2×10^6 and 6×10^6 T cells per mouse) into T cell-deficient, athymic nude mice, 1 day before R36A immunization. Purified CD4$^+$ and CD8$^+$ T cells had ≤ 1–2% cross-contaminating T cells as determined by flow cytometry (data not shown). In this regard, purified CD4$^+$ and CD8$^+$ T cells were mixed with 1 mg/mouse of anti-CD8 mAb or anti-CD4 mAb, respectively, to deplete any residual contaminating T cells upon in vivo transfer. Athymic nude mice, injected with R36A alone, demonstrated no detectable IgG anti-PspA, and a strongly reduced IgG anti-PC, response (Fig. 2). Transfer of CD4$^+$, but not CD8$^+$, T cells to athymic nude mice led to a significant induction of IgG PspA-specific titers, comparable to what is typically observed using control euthymic mice. Likewise, transfer of CD4$^+$ T cells led to a substantial up-regulation in the IgG, but not IgM, anti-PC response. The IgM anti-PC response is typically not reduced or only modestly reduced in mice lacking T cells relative to control mice (28). In contrast to the anti-PspA response, transfer of CD8$^+$ T cells induced a modest increase in IgG anti-PC titers that variably reached statistical significance, but this effect was strikingly lower on a per cell basis than that observed for the IgG anti-PC response after transfer of CD4$^+$ T cells. These data indicate that although CD8$^+$ T cells may play some role in stimulating in vivo IgG anti-PC responses, CD4$^+$ T cells are significantly more efficient helpers.

The T cell help for the IgG anti-PC response is TCR-nonspecific, but B7-dependent

Although polysaccharides do not associate with MHC molecules (3), thus typically precluding TCR-specific (cognate) T cell recognition of such Ags, the coexpression of polysaccharides with proteins by an intact pathogen could allow for cognate T cell help for polysaccharide-specific B cells, similar to the mechanism underlying the effectiveness of soluble protein-polysaccharide conjugate vaccines (16, 17). Additionally, direct TCR recognition of polysaccharide Ags, independent of MHC molecules, has been described (35, 36). To determine whether specific TCR recognition was required for T cell help for the IgG anti-PC response, we used a mouse that expressed a single TCR specificity for an Ag not present within R36A. Because all TCR transgenic mice exhibit variable degrees of endogenous TCR gene rearrangements leading to a T cell repertoire with multiple specificities, we used a TCR transgenic mouse (specific for the male, H-Y Ag in association with MHC class I) that had previously been crossed with mice

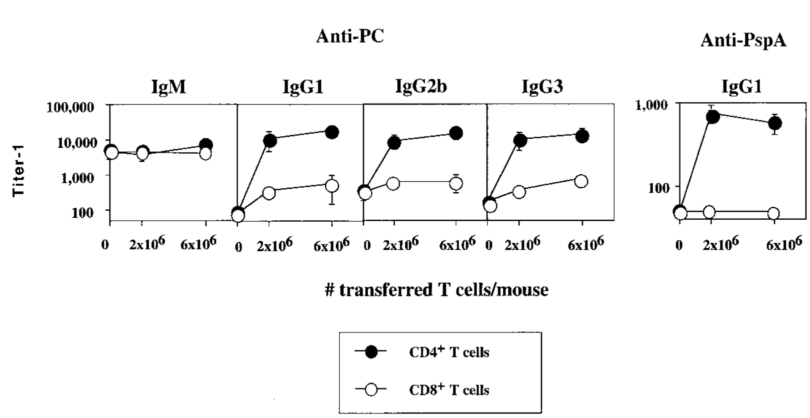

FIGURE 2. CD4$^+$ T cells are more efficient than CD8$^+$ T cells for stimulating an anti-PC response to R36A. Purified CD4$^+$ or CD8$^+$ T cells (2×10^6 or 6×10^6/mouse) were injected i.v. into athymic nude mice (five mice per group) followed 1 day later by i.p. immunization with R36A. CD4$^+$ T cells were mixed with 1 mg of anti-CD8 mAb and CD8$^+$ T cells were mixed with 1 mg of anti-CD4 mAb before injection. Sera were obtained on day 7 (anti-PC) and day 14 (anti-PspA) for determination of Ag-specific Ig isotype titers by ELISA. One of two representative experiments.

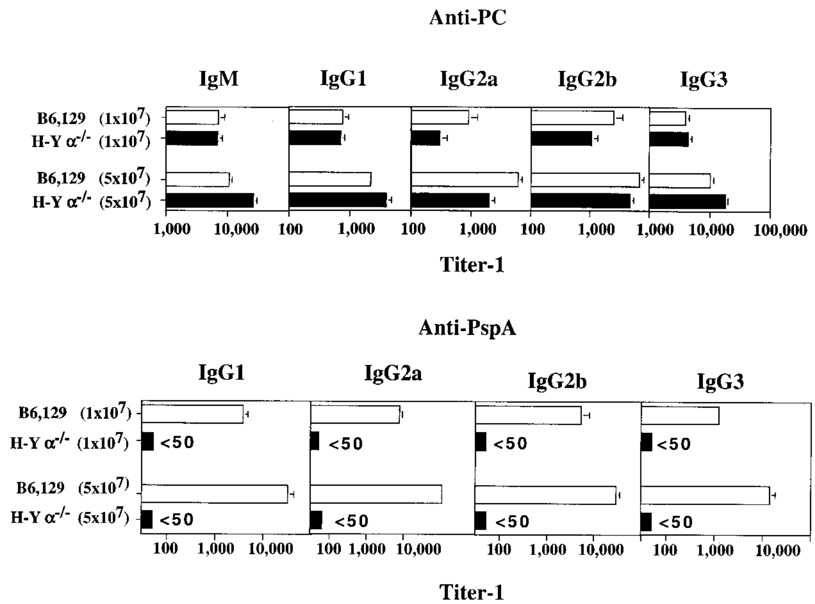

FIGURE 3. The T cell help for the IgG anti-PC response is TCR-nonspecific. H-Y $\alpha^{-/-}$ and control B6,129 mice (five mice per group) were injected i.p. with a suboptimal (1×10^7 CFU/mouse) or optimal (5×10^7 CFU/mouse) dose of R36A. Sera were obtained on day 7 (anti-PC) and day 14 (anti-PspA) for determination of Ag-specific Ig isotype titers by ELISA. One of three representative experiments.

genetically deficient in TCR-α (designated H-Y $\alpha^{-/-}$) (30). These mice expressed no endogenous TCR, possessed relatively normal numbers of CD4$^+$ and CD8$^+$ T cells, and had normal numbers of B cells. CD8$^+$ T cells in H-Y $\alpha^{-/-}$ mice expressed specificity for H-Y, whereas CD4$^+$ T cells were idiotype-negative, but did express TCR-β, perhaps in association with another undetermined protein. Importantly, although both the idiotype-positive and -negative T cells from H-Y $\alpha^{-/-}$ mice had functional TCR, as indicated by responsiveness to Con A, they failed to respond to a conventional protein Ag in adjuvant. This observation is consistent with other reports using nontransgenic TCR-$\alpha^{-/-}$ mice which also demonstrate complete abrogation of Ig responses to conventional protein Ags (37, 38). H-Y $\alpha^{-/-}$ and nontransgenic wild-type mice were thus immunized with optimal and suboptimal doses of R36A, and serum titers of IgG anti-PC and IgG anti-PspA isotypes were measured on the indicated days. As shown in Fig. 3, H-Y $\alpha^{-/-}$ mice elicited no detectable primary anti-PspA response of any IgG isotype after immunization with either optimal or suboptimal doses of R36A. Secondary challenge with R36A also failed to elicit a detectable anti-PspA response (data not shown). In contrast, R36A-induced titers of IgG anti-PC of all isotypes were essentially equivalent in H-Y $\alpha^{-/-}$ and control mice using either optimal or suboptimal doses of R36A (Fig. 3). Whereas, R36A immunization of control mice induced a significant germinal center reaction, no detectable germinal centers were observed in either unimmunized or immunized H-Y $\alpha^{-/-}$ mice (Fig. 4) consistent with the lack of response of H-Y $\alpha^{-/-}$ mice to immunization with conventional protein Ag (30) and our previous demonstration of a lack of memory generation for the IgG anti-PC response (28, 29). The elicitation of Ig-secreting cells in wild-type mice, outside of the germinal center environment following immunization with either protein or polysaccharide Ag, is well described (39, 40).

In the next set of experiments, we wished to determine whether the IgG anti-PC response to R36A in H-Y $\alpha^{-/-}$ mice was in fact T cell-dependent, as previously observed in control mice (28), and whether these T cells also required B7-dependent costimulation to mediate helper activity. Thus, H-Y $\alpha^{-/-}$ and control mice were injected with a combination of anti-CD4 + anti-CD8 mAbs or control rat IgG, 1 day before R36A immunization. As illustrated in Fig. 5*A*, depletion of T cells resulted in a complete abrogation of the anti-PspA response in wild-type mice. Although T cell depletion had no significant effect on the IgM anti-PC response either in H-Y $\alpha^{-/-}$ or wild-type mice, PC-specific IgG isotypes were significantly and comparably reduced in T cell-depleted H-Y $\alpha^{-/-}$ and wild-type mice, relative to mice treated with control rat IgG. Likewise, H-Y $\alpha^{-/-}$ mice treated with CTLA4Ig showed a significant reduction in PC-specific IgG isotypes, although no change in the IgM anti-PC response (Fig. 5*B*), consistent with previous data using wild-type mice (28). In an additional experiment, H-Y $\alpha^{-/-}$ mice were injected separately with either anti-CD4, anti-CD8, or control mAb, and the anti-PC response was measured 7 days after R36A immunization (Fig. 6). A substantial reduction (3- to 5-fold) in the IgG, but not IgM, anti-PC response, relative to mice treated with control mAb, was observed in anti-CD4-injected H-Y $\alpha^{-/-}$ mice, with only a modest reduction in IgG anti-PC titers observed in mice treated with anti-CD8. Collectively these experiments strongly suggest that, unlike the anti-PspA response, the CD4$^+$ T cell help for the anti-PC response is TCR-nonspecific, but B7-dependent.

Cathepsin S$^{-/-}$ mice have a defective anti-PspA, but normal anti-PC, response

Cognate interactions between APCs and T cells require APC processing of protein Ags into peptides, loading of peptides onto MHC molecules, and subsequent transport of peptide-MHC complexes to the APC surface for presentation to T cells. APCs that

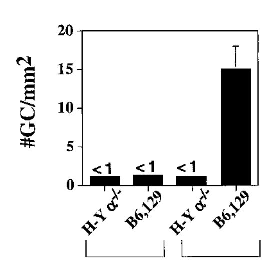

FIGURE 4. R36A induces germinal center formation in wild-type, but not H-Y $\alpha^{-/-}$, mice. H-Y $\alpha^{-/-}$ and control B6,129 mice (three mice per group) were injected i.p. with R36A and spleens were removed 14 days later for fluorescence immunohistochemistry to enumerate germinal center formation. One experiment.

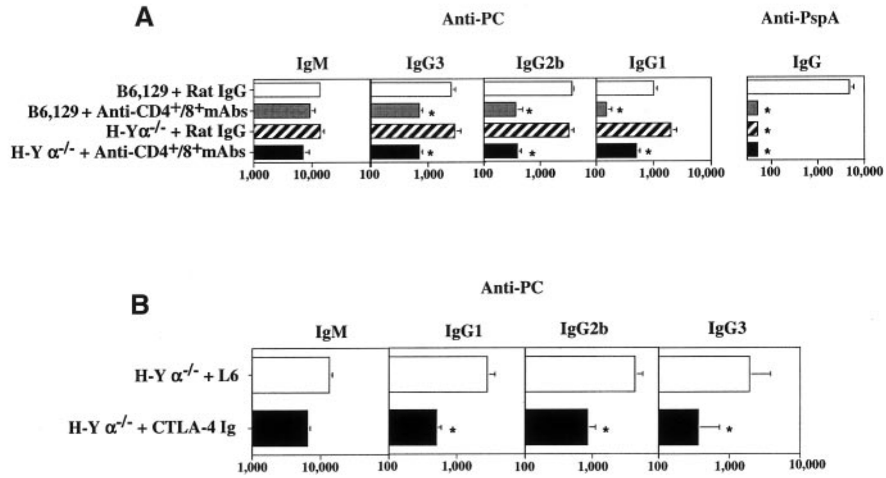

FIGURE 5. The IgG anti-PC response in H-Y $\alpha^{-/-}$ mice is T cell- and B7-dependent. *A*, B6,129 and H-Y $\alpha^{-/-}$ mice (five mice per group) were injected i.v. with either 1 mg each of anti-CD4 and anti-CD8 mAb or 2 mg of control rat IgG, followed 1 day later by i.p. immunization with R36A. *B*, H-Y $\alpha^{-/-}$ mice (five mice per group) were injected i.v. with either 0.2 mg of CTLA4Ig or control L6, followed 1 day later by i.p. immunization with R36A. Sera were obtained on day 7 (anti-PC) and day 14 (anti-PspA) for determination of Ag-specific Ig isotype titers by ELISA. One of two representative experiments each.

lack the protease cathepsin S are impaired in their ability to process the MHC class II-associated invariant chain beyond a 10-kDa fragment, resulting in a delay in peptide loading (41). Cathepsin $S^{-/-}$ mice were previously shown to contain normal numbers of B and T cells, but were significantly defective in specific IgG responses to protein Ag in adjuvant (31). Using cathepsin $S^{-/-}$ mice, we wished to test the hypothesis that if the T cell help for the IgG anti-PC response was TCR-nonspecific, it would not necessitate the acute processing of bacterial protein for MHC-dependent presentation. As anticipated, cathepsin $S^{-/-}$ mice showed a strong reduction in both the primary and secondary IgG anti-PspA response to R36A relative to wild-type mice (Fig. 7). In contrast, the lack of cathepsin S had no significant effect on the IgG anti-PC response consistent with the TCR-nonspecific nature of the T cell help for induction of PC-specific IgG.

Discussion

Collectively, these data indicate that the T cell help for the IgG anti-PC response to intact *S. pneumoniae*, in contrast to the IgG anti-PspA response, is more rapid, TCR-nonspecific, and not apparently dependent upon processing of new peptide-MHC class II complexes. Nevertheless, both the IgG anti-PC and anti-PspA responses depend on $CD4^+$ T cells and B7-dependent costimulation. These data are consistent with our recent observation that *S. pneumoniae*-pulsed DCs obtained from mice genetically deficient in MHC class II molecules, when adoptively transferred into naive wild-type mice, stimulate a normal IgG anti-PC response relative to control DCs, with the exception of IgG1 which is slightly reduced (42). In contrast, MHC class II-negative DCs, unlike wild-type DCs, fail to induce a detectable IgG anti-PspA response. Both the IgG anti-PC and anti-PspA responses to *S. pneumoniae*-pulsed wild-type DCs are abrogated by acute depletion of T cells (42).

The parameters that mediate the TCR-nonspecific help for the IgG anti-PC response are currently under investigation. The requirement for B7-dependent costimulation suggests that these T cells express CD28 and physically interact with B cells, macrophages, and/or DCs expressing B7. In this regard, *S. pneumoniae*-pulsed DCs derived from mice doubly deficient in B7-1 and B7-2, while failing to induce an in vivo IgG anti-PspA response, stimulate a normal IgG anti-PC response (42). Nevertheless, this IgG anti-PC response is still blocked by injection of CTLA4Ig, suggesting that either B7-expressing macrophages and/or B cells can stimulate TCR-nonspecific T cells for augmentation of the IgG anti-PC response.

Optimal B7-dependent costimulation typically requires up-regulation of B7 on the APC. In response to *S. pneumoniae*, this will likely occur through Toll-like receptor-mediated signaling via bacterial DNA, peptidoglycan, and/or lipoteichoic acid (43). B7 up-regulation will occur in concert with APC release of multiple cytokines that could further costimulate T cells. The subthreshold TCR-mediated signaling that T cells receive through continual recognition of self peptide-MHC complexes (44, 45), in concert with B7-dependent and cytokine-mediated costimulation, may trigger sufficient T cell effector function for stimulation of the IgG anti-PC response. A recent in vitro study demonstrates that even certain

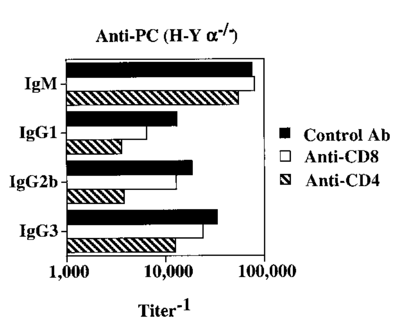

FIGURE 6. The IgG anti-PC response in H-Y $\alpha^{-/-}$ mice is largely dependent on $CD4^+$ T cells. H-Y $\alpha^{-/-}$ mice (three each) were injected i.v. with 1 mg of either anti-CD4, anti-CD8 mAb, or control rat IgG. Sera from each group were obtained on day 7, and pooled for determination of PC-specific Ig isotype titers by ELISA. One experiment.

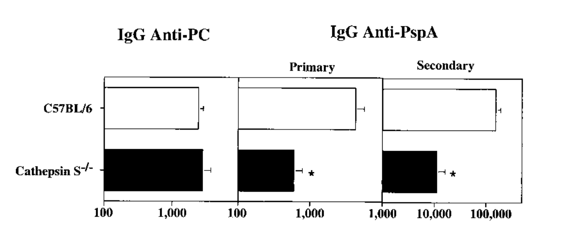

FIGURE 7. Cathepsin $S^{-/-}$ mice have a defective anti-PspA, but normal anti-PC response. Cathepsin $S^{-/-}$ and control C57BL/6 mice (five mice per group) were injected i.p. with R36A. Mice were boosted i.p. with a similar dose of R36A, 14 days after primary immunization. Sera were obtained 7 days (anti-PC), 14 days (primary anti-PspA), and 21 days (secondary anti-PspA) after primary immunization with R36A for determination of Ag-specific IgG titers by ELISA. One of two representative experiments.

combinations of cytokines alone can induce naive T cell proliferation, in the absence of TCR signaling (46).

It is likely that PC-specific B cells are strongly activated through multivalent mIg cross-linking by the repetitive PC moieties on the bacterial cell wall C-polysaccharide. This type of signaling would be expected to induce B cell proliferation alone, but in the presence of appropriate help, would costimulate Ig secretion and class switching (5, 6). TCR-nonspecific T cells, once activated, could provide this help either directly or indirectly. Thus, release of cytokines by these activated T cells either in contact with, or in close proximity to, mIg-activated PC-specific B cells could augment the IgG anti-PC response. Additionally, induction of TNF/TNFR family members on the surface of activated T cells, in contact with PC-specific B cells, could further costimulate B cell activation. Activated T cells could also stimulate APCs and other immune cells to release cytokines that might further amplify the B cell Ig response to bacterial challenge.

The rapid kinetics of the anti-PC, and other anti-polysaccharide Ig responses to extracellular bacteria may represent a distinct pathway of humoral immunity that contributes to early host protection against a rapidly dividing pathogen. The rapidity of this response may reflect the usage of distinct B cell subpopulations, such as B1 and marginal zone B cells (47–49), B cell tissue localization at sites of Ag entry, the potent mIg signaling in B cells mediated by polysaccharides expressing regularly spaced repeating identical antigenic epitopes (4), B cell helper activity of innate immune cells such as macrophages, DCs, and NK cells (6–15), and as described in this study, the rapid recruitment of $CD4^+$ Th cells. The accelerated kinetics of this T cell help may in part result from the TCR-nonspecific nature of the activational event, which would not necessitate the additional time required for Ag processing and presentation. Despite the TCR-nonspecific nature of this help, a level of specificity could be conferred by a requirement to strongly activate the polysaccharide-specific B cells through multivalent mIg cross-linking, which would then make B cells responsive to the action of cytokines and other forms of help (6).

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